

Exhibit A

Study of the activity of neutralizing FGF-23 of anti FGF-23 antibodies, 2C3B antibody and 2A2B antibody

The study was carried out by an assay in HEK293 cells expressing klotho proteins using EGR1 promoter/luciferase.

1. Production of mouse EGR1 promoter/luciferase fusion gene

A mouse EGR1 promoter/luciferase fusion gene was produced to examine the increase of mouse EGR1 gene expression. The sequences of the promoter was obtained by cloning a cDNA having the sequence from -1023 nucleotide of mouse EGR1 promoter to mRNA initiation site (+1) based on the descriptions of Christy et al., *Proc. Natl. Acad. Sci. USA* Vol. 85, pp.7857-7861, 1988 and the cloned cDNA was inserted into the plasmid, pGL4.20 (Promega). The constructed plasmid is referred to as mEGR1 promoter-luc./pGL4.20 hereinafter. The plasmid makes it possible to induce the expression of luciferase protein under the control of mouse EGR1 promoter and it has a puromycin resistant gene as a drug resistant maker gene.

2. Increase of luciferase activity induced by the stimulation of human FGF-23 in the klotho expressing HEK293 cells using mouse EGR1 promoter/luciferase reporter gene

The mouse EGR1 promoter/luciferase reporter gene constructed as described in paragraph 1 above was used to examine the change in EGR expression induced by the stimulation of FGF-23 in the human klotho expressing HEK293 cells. First, HEK293 cells were co-transfected with mEGR1 promoter-luc./pGL4.20 and hklotho/pLP-CMV-neo which is a vector constructed by inserting human klotho gene into expression vector, pLP-CMV-neo (Clonetech). Then, the cells were cultured in a selection medium containing puromycin and G418 and the two genes were stably introduced into the cells. After the cells were cultured on an appropriate plate for 24 hours, 10 ng/ml of purified recombinant human FGF23 (rhFGF23) and 10 µg/ml of heparin (Sigma) were added to the plate. Incubated at 37°C for 24 hours, luciferase activity was measured using Steady-Glo Luciferase assay system (Promega) according to the accompanying protocol. TopCount™ (Packard) was used for the measurement. As a result, the expression of luciferase was induced depending on rhFGF23.

3. Effects of anti FGF-23 antibody in the assay system using klotho protein expressing HEK293

The activity of neutralizing FGF-23 of 2C3B antibody and 2A2B antibody was studied according to the method described in paragraph 2 above. Just before the addition of FGF-23, the antibody was added at a concentration of 1 or 10 μ g/ml. As figures 1 and 2 show, 2C3B inhibited the increase of luciferase activity by FGF-23 at a low concentration of 1 μ g/ml, while 2A2B antibody did not inhibit the increase of luciferase activity at a high concentration of 10 μ g/ml.